

SECTION ON MICROBIOLOGY

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I. EXECUTIVE SESSION

Reading of the Minutes

II. PAPERS OF THE EVENING

INSECTICIDES

a. Chemical aspects of some of the newer insecticides

H. L. Haller (by invitation)
U. S. Department of Agriculture
Washington, D. C.

GREGORY SHWARTZMAN
Chairman

b. The major toxic actions of insecticides

A. J. Lehman (by invitation)
Federal Security Agency, Food
and Drug Administration,
Washington, D. C.

c. Recent advances in medical and veterinary entomology

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HARRY MOST
Secretary

Chemical Aspects of Some of the Newer Insecticides

H. L. HALLER

The use of chemicals to control injurious insects has been accorded more publicity during the past five years than in all the previous years during which research on insecticides has been carried out. This attention has been due in a large measure to the discovery of the outstanding insecticidal properties of several synthetic organic compounds, especially chlorinated hydrocarbons, such as DDT, benzene hexachloride, chlordane, and toxaphene (chlorinated camphene). More recently several organic phosphorus compounds have been found to be highly toxic to a wide range of insects. Several of the discoveries were made at a time when the need for better insecticides to control insect pests on food and fiber crops, stored products, livestock, and forest products was greater than ever before and when the available supply of the standard agricultural and household insecticides—pyrethrum, nicotine, rotenone, and the ar-

senicals—was unusually low. Although the findings may appear to some to be due to hastily initiated wartime research and largely fortuitous, this is not the case. Rather, the discoveries are the culmination of extensive explorations over a period of about 80 years. Chemicals have been used for a longer time to combat injurious insects but it is only since about 1867 that a systematic study to find new insecticides has been under way.

The early studies were concerned largely with inorganic compounds such as paris green and lead arsenate. By 1910 agricultural practices had become more intensified and insect populations increased, as did the losses which they caused to many important crops. About the same time investigators became interested in finding out how insecticides kill, and emphasized the importance of their physical characteristics, such as wetting and spreading of sprays, on

foliage and insects. A few years later studies were initiated to determine the chemical structures of the highly effective, naturally occurring plant insecticides, the pyrethrins and rotenone. These studies proved fruitful, and the structural formulas that were developed served as patterns for the preparation of organic compounds thought to be toxic to injurious insects. The studies were supplemented by the testing of a large number of synthetic organic compounds which became available soon after the first World War and for which a use was being sought. The two approaches have been profitable and have led to the discovery of a number of compounds highly toxic to insect pests.

The knowledge that an organic compound is highly toxic to several species of insects is not the only requirement for its immediate widescale use. Before its utility in controlling injurious insects can be determined, information must be obtained as to the safety of the chemical to public health, to farm animals, to soils, to vegetation, to beneficial insects including bees, and to wildlife wherever these interests are involved. It is necessary to know the range of effectiveness (that is, to what kinds of insects the product is toxic); the stage of the insect that is destroyed (egg, larva, or adult). Such studies are the joint affair of biologists and chemists.

Few, if any, of the newer insecticidal chemicals are suitable for use as insecticides in the undiluted form. Aside from the fact that it would be uneconomical for most purposes to use potent agents without diluting them greatly, the physical properties of the substances usually are such that they are unsuited for direct application. To determine whether the compound can best be applied as a dust, spray, or as an aerosol a knowledge of the physical and chemical characteristics of the product is needed. Physical and chemical studies should include determination of the composition of the product, its solubility in various solvents, and its compatibility with other insecticides, fungicides, and dust diluents. Methods for the analysis of the product itself, as well as in dusts, in sprays, and in combination with other insecticides, must

be developed. Procedures for determining the product as a spray residue are also needed.

As the time allotted to me will not permit the discussion of all of the newer insecticides I shall confine my talk to the chemistry of some of the more important chlorinated hydrocarbons and the organic phosphorus compounds.

The chlorinated hydrocarbons that have attracted most attention are DDT, TDE (also known as DDD), methoxychlor (methoxy analog of DDT), benzene hexachloride, toxaphene (chlorinated camphene), and chlordane. Although methoxychlor contains oxygen it is included because of its close relationship to DDT. The technical grades of all six of these products are mixtures of two or more isomeric compounds, the relative insecticidal value of which varies with the product and the test insect. The empirical formula and the total chlorine content of each is given in Table I.

These products possess one common chemical characteristic to which their outstanding insecticidal property has been attributed. In the presence of alcoholic alkali and in some cases with traces of certain metals, such as iron, aluminum and others they lose one or more moles of hydrogen chloride. If this reaction is a factor in making them toxic to insects it is only a minor one as many related compounds are known which likewise yield

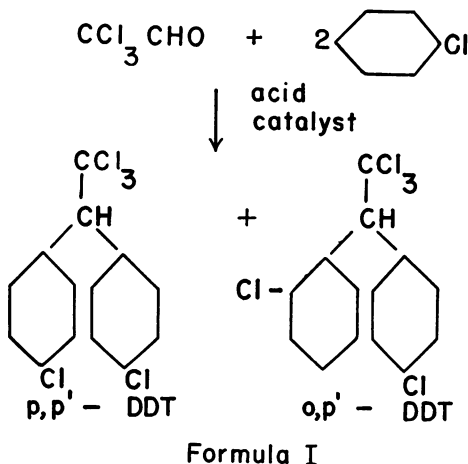
TABLE I.
FORMULA AND CHLORINE CONTENT OF SOME
CHLORINATED HYDROCARBON INSECTICIDES

| <i>Product</i> | <i>Formula</i> | <i>% Chlorine</i> |
|---------------------------------------------|-----------------------|-------------------|
| DDT | $C_{14}H_9Cl_5$ | 50.01 |
| TDE | $C_{14}H_{10}Cl_4$ | 44.33 |
| Methoxychlor | $C_{16}H_{15}O_2Cl_3$ | 30.77 |
| Benzene hexachloride | $C_6H_6Cl_6$ | 73.15 |
| Chlordane | $C_{10}H_6Cl_8$ | 69.22 |
| Toxaphene (chlor- inated camphene) | $C_{10}H_{10}Cl_8$ | 68.54 |

hydrogen chloride but are relatively non-toxic to insects. The speed at which the hydrogen chloride is released from the compound is not a vital factor either because DDT yields it quite rapidly whereas the methoxy analog of DDT does not.¹ Our knowledge of the relationship between insecticidal action and chemical constitution is too limited to state that any one reaction will determine whether a compound possesses insecticidal properties.

DDT

The studies leading to the discovery of DDT as an insecticide are presented in detail by Lauser, Martin and Muller.² The symbol DDT is a contraction for Dichloro-Diphenyl-Trichloroethane. However, the term DDT has been confined to the product obtained on condensation of chloral with chlorobenzene in the presence of sulfuric acid. The chemistry of DDT has been investigated more extensively than that of the other chlorinated hydrocarbons.³ Formula I illustrates the formation of DDT.



When pure chloral and pure chlorobenzene are used the reaction product consists essentially of two isomeric compounds, p,p'-DDT and the o,p'-DDT. Technical DDT contains approximately 3 to 4 parts of the former isomer to one part of the latter.

The toxicity of these two isomers to several insects is shown in Table II.

TABLE II.
COMPARATIVE TOXICITY OF P,P'-O,P'-DDT TO
VARIOUS INSECTS*

| Insect | <i>p,p'</i> -DDT | | <i>o,p'</i> -DDT | |
|------------------|-------------------------|-----------|-------------------------|-----------|
| | Concen- tration % | Kill % | Concen- tration % | Kill % |
| Houseflies | 1.0 | 70 | 5.0 | 1 |
| Body lice | 0.05 | 100 | 1.0 | 0 |
| Mosquitoes† | | | | |
| Adults | 0.5 | 49 | 5.0 | 23 |
| Larvae | 0.0015 | 50 | 0.011 | 50 |
| | (p.p.m.) | | (p.p.m.) | |

* These results were obtained at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine.

† *Anopheles quadrimaculatus* Say.

It will be noted that although the o,p'-DDT is practically non-toxic to the adult form of these insects it is a very effective compound against mosquito larvae. This specificity manifests itself with many of the synthetic organic compounds.

It is natural to inquire as to the effect of replacing one or more chlorine atoms in DDT in both the benzene rings and the ethane part of the molecule with other atoms or radicals. Such compounds have been synthesized but more work has been done with analogs in which the chlorine atoms of the benzene rings have been replaced than with compounds in which the chlorine atoms in the ethane molecule have been substituted.

The toxicity of some analogs of DDT to mosquito larvae is given in Table III.

It is of interest to note that the replacement of chlorine atoms in the benzene ring by the hydroxyl radical gives a product that is relatively non-toxic. Methylation of the free hydroxyl group produces a potent insecticide. The product obtained with the methyl radical in place of chlorine is effective but that with the tertiary butyl group is of little value as an insecticide. Similar results were obtained when these

TABLE III.

TOXICITY TO FOURTH-INSTAR LARVAE OF ANOPHELES QUADRICIMACULATUS OF DDT ANALOGS IN WHICH CHLORINE ATOMS IN BENZENE RINGS HAVE BEEN REPLACED WITH OTHER ATOMS OR RADICALS*

| <i>Substituents on Diphenyldichloroethane</i> | <i>Dosage, P.P.M.</i> | <i>% Mortality after 48 Hr.</i> |
|-----------------------------------------------|-----------------------|---------------------------------|
| p,p'-di-Cl (DDT) | 0.005 | 100 |
| p,p'-di-Br | 0.005 | 100 |
| p,p'-di-F | 0.01 | 85 |
| p,p'-di-CH ₃ O | 0.01 | 100 |
| p,p'-di-OH | 10 | 20 |
| p,p'-di-H | 0.1 | 25 |
| p,p'-di-CH ₃ | 0.01 | 100 |
| p,p'-di-tert-Butyl | 10 | 20 |
| p,p'-Cl,H | 0.01 | 85 |

* Deonier, C. C., Jones, H. A., Haller, H. L., Hinchey, E., and Incho, H. H., *Soap and Sanitary Chemicals*, 1946, 22:118.

compounds were tested against a number of agricultural insect pests.^{4,5}

Although p,p'-DDT is effective against a wider range of injurious insects than most of the other synthetic organic insecticides so far tested, it is not a panacea for all ills due to insects. DDT has little or no effect on the boll weevil, the cotton leafworm, the cotton aphid, the Mexican bean beetle, red spiders, cattle grubs, adults of the Florida and California red scales, the sugar-cane borer, orchard mites, the parlatoria date scale, and the plum curculio. It is effective against some aphids but as a rule is less effective than nicotine. It has also registered failures for one reason or another against the tobacco hornworm, the cabbage seedpod weevil, the tomato russet mite, etc.

METHOXYCHLOR

Methoxychlor is the coined name adopted for the methoxy analog of DDT, 1,1,1-trichloro - 2,2 - bis(*p* - methoxyphenyl)ethane. The technical product is obtained on condensation of chloral and anisole.⁶ Methoxychlor is effective against numerous agricul-

tural insect pests, including the Mexican bean beetle, and has been reported⁷ to give a more rapid "knockdown" of flies than DDT.

TDE (DDD)

TDE also called DDD is the symbol for dichloro diphenyl dichloroethane, a tetra chloro diphenyl ethane. *p,p'*-TDE is 1,1-dichloro - 2,2 - bis(*p* - chlorophenyl)ethane. TDE is obtained on condensation of dichloroacetaldehyde and chlorobenzene.⁸ The *p,p'*-TDE is equal in toxicity to *p,p'*-DDT against mosquito larvae⁹ and closely parallels DDT in effectiveness against both household and agricultural insect pests.⁸

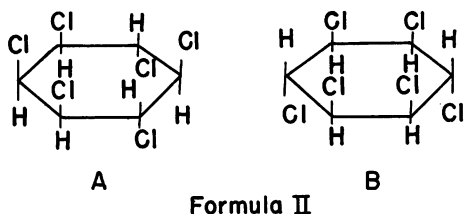
BENZENE HEXACHLORIDE

The insecticidal properties of benzene hexachloride or 1,2,3,4,5,6-hexachlorocyclohexane were discovered independently about the same time in France¹⁰ and England¹¹ during World War II while there was no communication between the two countries.

It is of interest that benzene hexachloride C₆H₆Cl₆ is insecticidal whereas hexachlorobenzene, C₆Cl₆ has no insecticidal properties.

Benzene hexachloride is prepared by the addition of chlorine to benzene in the presence of actinic light.¹² The reactants are required in the ratio of 3 moles of chlorine to one mole of benzene. It has not yet been possible to add chlorine to the three double bonds stepwise, that is, with the formation of dichloro or tetrachloro derivatives. When the ratio is other than three to one, the final product under all conditions so far tried, is a mixture of unchanged benzene and benzene hexachloride. The reaction product consists of a mixture from which five isomeric benzene hexachlorides have been isolated. These have been designated as alpha, beta, gamma, delta, and epsilon isomers. Their alphabetical designations indicate the order in which they have been discovered and described in the literature and bear no relation to their relative structures. Of these isomers only one, the gamma isomer, which occurs to the extent of 10-12 per cent in technical benzene hexachloride, has outstanding insecticidal properties.

The formation of the isomers may be explained in the following manner. If it is assumed that all of the carbon atoms of the cyclohexane ring lie in one plane eight stereoisomers of hexachlorocyclohexane, one of which exists in mirror-image form, are theoretically possible. The configuration shown in Formula IIa was originally assigned to the gamma isomer. This configuration has recently been shown to be incorrect and the isomer may be more nearly correctly represented by Formula IIb.¹³



Benzene hexachloride is toxic to a wide range of agricultural insect pests, especially the cotton boll weevil against which DDT is ineffective. The technical product has a musty persistent odor which may limit its usefulness for certain purposes.

Table IV shows the toxicity of each of four isomers to mosquito larvae. Like p,p'-DDT, the gamma isomer is slow in its killing action and does not cause "knockdown." At the time that these tests were made the epsilon isomer was not available.

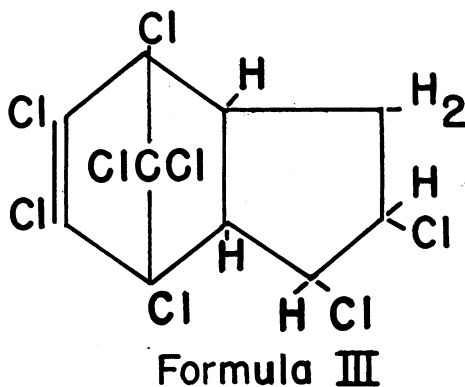
CHLORDANE

Chlordane is the common name assigned to the compound designated as 1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindan and having the structure shown in Formula III.

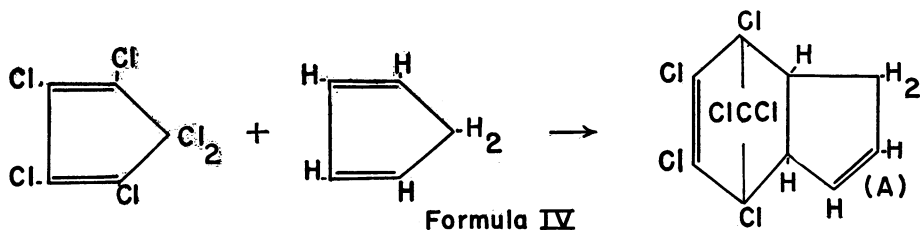
It is prepared by allowing hexachlorocyclopentadiene and cyclopentadiene to react as shown in Formula IV.

TABLE IV—TOXICITY OF VARIOUS ISOMERS OF BENZENE HEXACHLORIDE TO LARVAE OF ANOPHELES QUADRIMACULATUS

| Isomer | Dosage P.p.m. | Mortality in | |
|-------------|------------------|---------------------|---------------------|
| | | 24 Hours Percent | 48 Hours Percent |
| Alpha | 2.5 | 88 | 92 |
| Beta | 100 | 22 | 40 |
| Gamma | .01 | 80 | 100 |
| Delta | 2.5 | 40 | 62 |
| DDT | .01 | 98 | 100 |
| | .005 | 53 | 85 |



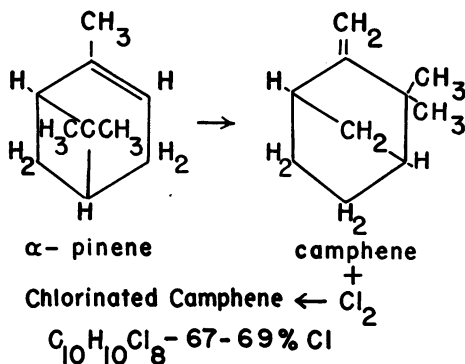
The adduct IV, dissolved in a suitable solvent, such as carbon tetrachloride, is treated with chlorine gas with the resultant addition of 2 atoms of chlorine to the double bond (A) to form chlordane (III).¹⁴ Technical chlordane contains 60-75 per cent of chlordane, the remainder being closely related compounds that occur in the normal process of manufacture and which are toxic to insects. Chlordane has been found to be toxic to a wide range of agricultural and



household insect pests.¹⁵ It is especially effective against grasshoppers and cockroaches and it has considerable residual effectiveness.

TOXAPHENE (CHLORINATED CAMPHENE)

Another chlorinated hydrocarbon with outstanding insecticidal properties is found among the terpene derivatives. The product was originally designated a chlorinated bicyclic terpene.¹⁶ Subsequently the coined name chlorinated camphene was developed for it. More recently this has been changed to toxaphene by which the technical product is now known. By the chlorination of camphene, which is obtained by the isomerization of pinene from pine wood extract, to a chlorine content of 67-69 per cent a product is obtained which has the approximate empirical formula $C_{10}H_{10}Cl_8$. The probable method of formation is given in Formula V although the exact structure is not known.



Formula V

Toxaphene has been found toxic to a considerable number of agricultural insect pests, especially grasshoppers and insects attacking cattle.

ORGANIC PHOSPHORUS COMPOUNDS

Three organic phosphorus-containing compounds, hexaethyl tetraphosphate, tetraethyl pyrophosphate, and parathion have received considerable attention for the control of injurious insects. Hexaethyl tetraphosphate is the name given by a German in-

vestigator to a product obtained by allowing triethyl orthophosphate to react with phosphorus oxychloride as shown in Formula VI.



Formula VI

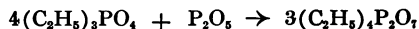
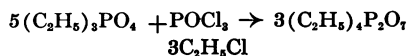
Essentially the same reaction product is obtained from triethyl orthophosphate and phosphorus pentoxide as indicated in Formula VII.



Formula VII

Chemical examination of hexaethyl tetraphosphate has shown it to be a mixture containing as its principal active ingredient the compound tetraethyl pyrophosphate. Other constituents are ethyl metaphosphate and triethyl orthophosphate, both of which are relatively insecticidally inactive materials. Pentaethyl triphosphate, an unstable ester possessing insecticidal properties, may also be present but this has not been established with certainty.

As produced by either of the reactions above tetraethyl pyrophosphate is present to the extent of about 15-20 per cent. Modification of either process by increasing the proportion of the triethyl orthophosphate results in a product of empirical formula $(C_2H_5)_6P_4O_{13}$, which is an even more potent insecticide than hexaethyl tetraphosphate. The reaction is shown in Formula VIII.



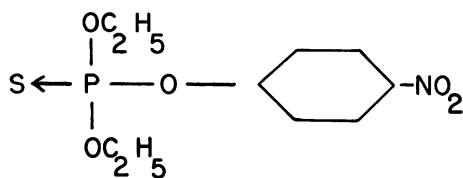
Formula VIII

The reaction products are not pure tetraethyl pyrophosphate but are mixtures of ethyl phosphates and polyphosphates containing approximately 40 per cent of this principal active ingredient.

German chemists had reported that in water solution or in the presence of atmospheric moisture hexaethyl tetraphosphate was completely hydrolyzed in 24 hours to ethyl alcohol and orthophosphoric acid. Hall and Jacobson¹⁷ in a study of the hydrolysis of hexaethyl tetraphosphate, however, found that although rapid hydrolysis to non-insecticidal products occurs, the end-products are monoethyl and diethyl orthophosphoric acid. Pure tetraethyl pyrophosphate hydrolyzes to diethyl orthophosphoric acid. This rapid hydrolysis to non-toxic products greatly limits the duration of the insecticidal effectiveness of tetraethyl pyrophosphate but it also eliminates the danger of toxic residues on the crops treated.

Both hexaethyl tetraphosphate and tetraethyl pyrophosphate are especially useful against mites and such soft-bodied insect pests as aphids, mealy bugs, and thrips. Both compounds have been shown to inhibit the action of cholinesterase¹⁸ and acetyl esterase.¹⁹

Another phosphorus-containing organic insecticide is O,O-diethyl O-p-nitro-phenyl thiophosphate Formula IX to which the coined name parathion has been assigned.



Formula IX

This insecticide also was discovered in Germany during World War II where it was designated E-605.^{20, 21}

According to Thurston²¹ the product is obtained by the following sequence of reactions: "Phosphorus trichloride is combined with sulfur by heating at 130°C. for two hours in a lead-lined autoclave. A quantitative yield of phosphorus thiotrichloride is obtained. The soluble sulfur compounds are removed by distilling the product from a lead-lined kettle. The resulting product boils at 125°/760 mm. pressure. This material is then combined with sodium ethox-

ide contained as a 10 per cent solution in alcohol. The formed diethoxythio-phosphorous chloride is poured into water and decanted. This intermediate product is stable in water and alcohol and only slowly decomposes when heated with them at temperatures of 80-90°C. Sodium p-nitrophenate in chlorobenzene is then combined with the diethoxy-thiophosphorous chloride by heating at 130°C. The finished product is not distilled, only the chlorobenzene, which is used as the reaction medium, is removed. The yield of the crude product is 80-90 per cent, since the formation of phosphorous thiotrichloride is quantitative (sic) and the remainder of the reactions are very good."

Parathion is an oily liquid with a disagreeable garlicky odor. It has a low vapor pressure of 0.0006 mm. of mercury at 24° and can be distilled only under greatly reduced pressure. It is not hydrolyzed appreciably in neutral water but is hydrolyzed in alkaline waters. It has proved highly toxic to all of a wide range of insects against which it has been tested. Since it is relatively stable, its effectiveness persists for a considerable time, reportedly from a few days to several weeks.

Both tetraethyl pyrophosphate and parathion are highly toxic to warm-blooded animals.²² They are absorbed through the skin and extreme care must be taken in using them. Moreover, in the case of parathion because of its greater stability there is the possibility of toxic residues from its application.

SUMMARY

Although much progress has been made in the development of new materials for combating insect pests many problems must be solved before their utility as practical control measures is determined. It is necessary to know against what kinds of insects the product is effective, the stage at which the insect is most susceptible—egg, larva, or adult—and the compatibility of the material with solvents, carriers, fungicides, or other insecticides. It must also be determined whether the material can be applied best as a dust, a spray or an aerosol and whether it causes plant injury when applied either to the foliage or to the soil. Its effect on

beneficial insects, such as bees and various parasites and predators, and its toxicity to warm-blooded animals, especially man, must also be ascertained. These and many other factors need to be established before a new product finds full use in the field of economic entomology.

R E F E R E N C E S

1. Cristol, S. J. Kinetic study of the dehydrochlorination of substituted 2,2-diphenylchloroethanes related to DDT, *J. Am. Chem. Soc.*, 1945, 67:1494.
2. Lugger, P., Martin, H. and Muller, P. Uber Konstitution und toxische Wirkung von naturlichen und neuen synthetischen insektentotenden Stoffen, *Helvet. chim. acta*, 1944, 27:892.
3. Haller, H. L., Bartlett, P. D., Drake, N. L., Newman, M. S. *et al.* Chemical composition of technical DDT, *J. Am. Chem. Soc.*, 1945, 67:1591.
4. Questel, D. D. and Gertler, S. I. U. S. Bur. Entomology and Plant Quarantine, E-612 (Processed).
5. Siegler, E. H. and Gertler, S. I. Toxicity of diaryl trichlorethanes and dichloroethylenes to codling moths, *J. Econ. Entomol.*, 1944, 37:845.
6. Bousquet, E. W. and Goddin, A. H. U. S. Patent 2,420,928, May 20, 1947.
7. Prill, E. A., Hartzell, A. and Arthur, J. M. Insecticidal activity of some alkoxy analogs of DDT, *Science*, 1945, 101:464.
8. Alsterlund, J. Rhothene D-3 in the pest control field, *Pests*, 1946, 14, no. 5:10.
9. Deonier, C. C. and Jones, H. A. TDE, 1,1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane, as anopheline larvicide, *Science*, 1946, 103:13.
10. Dupire, A. and Raucourt, M. *Compt. rend. Acad. agric. de France*, 1943, 29, No. 17:470.
11. Slade, R. The γ -isomer of hexachlorocyclohexane (gammexane), *Chemistry & Industry*, 1945, 40:314.
12. Hardie, T. U. S. Patent 2,218,148, Oct. 15, 1940.
Haller, E. L. and Bowen, C. V. Basic facts about benzene hexachloride, *Agricultural Chemicals*, 1947, 2, No. 1:15.
13. Van Vloten, G. W. *et al.* Crystal structure of "Gammexane," *Nature*, 1948, 162:771.
14. Hyman, J. Mexican Patent, 45,398, March 19, 1947.
15. Kearns, C. W. *et al.* A new chlorinated insecticide, *J. Econ. Entomol.*, 1945, 38:661.
16. Stearns, L. A., Parker, W. L. and Beacher, J. H. Progress report on a new insecticide, *Soap & Sanitary Chemicals*, 1947, 23, No. 1:117.
17. Hall, S. A. and Jacobson, M. Hexaethyl tetraphosphate and tetraethyl pyrophosphate, *Indust. & Engin. Chem.*, 1948, 40:694.
18. Dubois, K. P. and Mangun, G. H. Effect of hexaethyl tetraphosphate on choline esterase in vitro and in vivo, *Proc. Soc. Exper. Biol. & Med.*, 1947, 64:137.
19. Jansen, E. F., Fellows-Nutting, M.D. and Balls, A. K. Reversible inhibition of acetylcholinesterase by diisopropyl fluorophosphate and tetraethyl pyrophosphate, *J. Biol. Chem.*, 1948, 175:975.
20. Martin, H. and Shaw, H. B. I. O. S. *Final Report 1095*. U. S. Dept. of Commerce OTS, PB-L78244, 1946.
21. Thurston, J. T. F. I. A. T. *Final Report 949*. U. S. Dept. of Commerce OTS, PB-60890, 1946.
22. Mumford, S. A. and Perren, E. A. B. I. O. S. *Final Report 714*. U. S. Dept. of Commerce OTS, PB-L 87923R (no date).